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Certificate of Analysis

PP1 alpha

(Recombinant enzyme expressed in *E.coli* cells)
Item # 14-595, 14-595-K, 14-595M
Parent Lot # WAB0439

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: Untagged, recombinant, full length, human PP1 alpha expressed in *E.coli* cells. Purified using Heparin sepharose followed by Q sepharose. Purity 59% by SDS PAGE and Coomassie blue staining. MW = 37.6kDa.

Formulation: 0.63mg/ml of enzyme in 50mM Hepes/NaOH pH7.5, 300mM NaCl, 50% glycerol, 0.1mM EGTA, 1mM $MnCl_2$, 0.03% Brij-35, 0.1mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol. Liquid at -20°C.

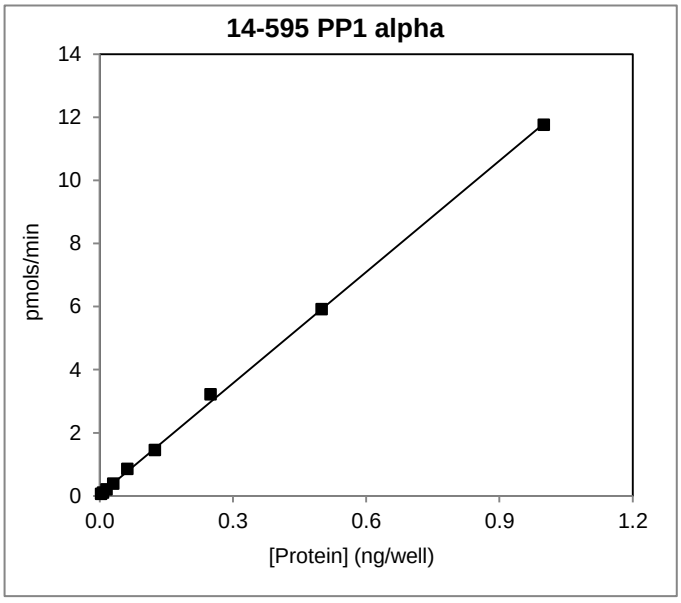
Specific Activity PP1a (Parent lot# WAB0439): 12373Units/mg. A Unit releases 1nmole of phosphate min^{-1} from the phosphorylated substrate DiFMUP (6,8-difluoro-4-methylumbelliferyl phosphate Molecular Probes cat# D6567.)

Storage and Stability: On receipt of material store at -20°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

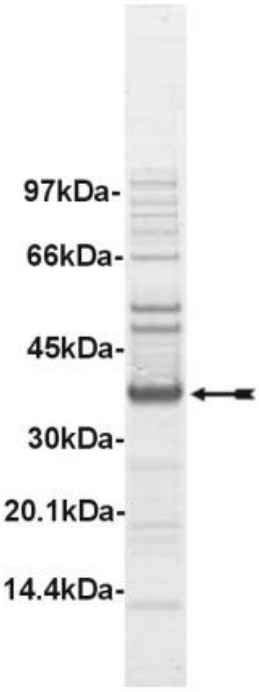
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Phosphatase Assay: Assay background was the 0 time point Fluorescence Intensity (FI) subtracted from the FI post 60 min incubation at room temperature (22°C). Quantification of FI was against a DiFMU std curve in the reaction buffer. The assay procedure followed is described overleaf.



MS Tryptic Fingerprint: Confirmed product identity as PP1 alpha with the translated sequence listed on page three.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of active PP1 alpha.

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Phosphatase Assay Protocol

Stock Solutions:

1. 1x Reaction Buffer: 57mM Hepes pH7.2, 10mM $MnCl_2$, 0.167mM DTT, 0.83% (v/v) glycerol, 0.0167% (w/v) BSA, 0.002% Brij 35.
2. 500µM DiFMUP (Molecular Probes Catalogue # D6567) in water.
3. 100mM sodium orthovanadate.
4. 500µM DiFMU (Molecular Probes Catalogue # D6566) in water for the calibration curve.

Assay Procedure:

1. Add 15µl PP1a diluted in 1x reaction buffer (0.004–1.0ng well)
2. Add 10µl DiFMUP 500µM stock solution (200µM final assay concentration).
3. Incubate for 30 minutes at room temperature.
4. Stop the reaction by adding 5µl of 100mM sodium orthovanadate.
5. Read FI using an appropriate reader (Excitation 340nm; Emission 450nm).
6. Subtract the zero enzyme values from each FI reading and calculate the enzyme activity by conversion to nmoles product formed using a DiFMU standard calibration curve.

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PP1 alpha Sequence Information

Protein human PP1alpha

Tags untagged

Native sequence M1 of the recombinant protein is equivalent to M1 of PP1 alpha

Accession number GenBank NM_002708. The PP1alpha protein sequence is identical to the human PP1 alpha described in GenBank NM_002708. The DNA sequence, however, is more closely related to the rabbit cDNA described in GenBank X14832.

Recombinant PP1 alpha amino acid sequence:

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  1 MSDSEKLNLD SIIGRLLEVQ GSRPGKNVQL TENEIRGLCL KSREIFLSQP ILLELEAPLK
 61 ICGDIHGQYY DLLRLFEYGG FPPESNYLFL GDYVDRGKQS LETICLLLAY KIKYPENFFL
121 LRGNHECASI NRIYGFYDEC KRRYNIKLWK TFTDCFNCLP IAAIVDEKIF CCHGGLSPDL
181 QSMEQIRRIM RPTDVPDQGL LCDLLWSDPD KDVQGWGEND RGVSFTFGAE VVAKFLHKHD
241 LDLICRAHQV VEDGYEFFAK RQLVTLFSAP NYCGEFDNAG AMMSVDETLM CSFQILKPAD
301 KNKGKYGQFS GLNPGGRPIT PPRNSAKAKK
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Recombinant PP1 alpha nucleotide sequence:

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  1 atgtccgaca gcgagaagct caacctggac tctatcatcg ggcgcctgct ggaagtgcag
 61 ggctcgcggc ccggaaagaa tgtgcagctg acggagaacg agatccgtgg tctgtgcctc
121 aaatcccggg agatcttcct gagccagccc attctgctgg agctggaggc gcccctcaag
181 atctgcggtg acatccacgg ccaatactac gacctgctgc ggctgttcga gtacggcggc
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301 ctggagacca tctgcctgct gctggcctac aagatcaagt accccgagaa cttcttcctg
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421 aagagacgct acaacatcaa gctgtggaag acgttcaccg actgcttcaa ctgcctgccc
481 atcgcggcca ttgtggacga gaagatattc tgctgccatg gcggcctctc ccccgacctg
541 cagtccatgg agcagatccg gcgcatcatg cggcccacgg acgtgcccga ccagggcctg
601 ctgtgtgacc tgctgtggtc tgaccccgac aaggacgtgc agggctgggg cgagaacgac
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Reviewed and approved by site quality representative.